REMARKS

The Office Action mailed July 2, 2002, has been received and reviewed. Claims 1-5, 10-13, 16-25, 31 and 32 are pending in the application. All claims stand rejected. Claims 1-5, 10-13, 16-25, 31 and 32 have been amended as set forth herein. All amendments are made without prejudice or disclaimer. Reconsideration is respectfully requested.

I. Disapproval of the Drawings

The drawings were objected to for not having uniformly thick and well defined lines and for not having plain and legible numbers and reference characters. Attached hereto is a Letter to the Chief Draftsman correcting the informalities. Withdrawal of the objections is thus requested.

II. Objection to the Specification

The specification was objected to for including a typographical error on page 7. The typographical error has been corrected. Withdrawal of the objection is thus requested.

III. Rejections under 35 U.S.C. § 112, first paragraph

A. Enablement

Claims 1-5, 10-13, 16-25 and 31-32 stand rejected under 35 U.S.C. § 112, first paragraph, as assertedly lacking enablement for how to make and use the invention commensurate in scope with the claims. At least partially in view of the amendments to claims 1-5, 10-13, 16-25 and 31-32, applicants respectfully traverse the rejections.

Specifically, it was thought that undue experimentation would be required for one skilled in the art to practice the claimed invention since the art was deemed unpredictable and the specification was thought to disclose a limited number of working examples. Although applicants do not agree that the claims lack enablement, the claims have been amended for the sake of expedited prosecution. As amended, the claims are directed to a "pharmaceutical composition" comprising a "peptide" and not to "any compound."

As described in the as-filed specification, Examples 2, 3 and 4 are working examples of *in vivo* experiments performed on sensitized mice. The disclosed experiments show a clear and

distinct effect of the ability of the claimed peptide to inhibit bronchoconstriction (*See*, Specification, page 9, lines 16-20) and the effects of the claimed peptide on ear-swelling (*Id.* at page 10, lines 11-15). The disclosed examples also indicate that the claimed peptide can be applied in different ways, that the claimed peptide was not subject to proteolytic cleavage before exhibiting the peptide's effects, that the claimed peptide reaches the target area, and that the claimed peptide did not have adverse side effects prohibitive to the use of the peptide as a pharmaceutical composition. (*See*, *Id.* at page 9, lines 16-19 and page 10, lines 12-14).

Accordingly, the disclosed examples illustrate that the binding of free IgLC to mast cells can be prevented using the peptide of the claimed invention. Reconsideration and withdrawal of the enablement rejections of claims 1-5, 10-13, 16-25 and 31-32 are thus requested.

B. Written Description

Claims 1-5, 10-13, 16-25 and 31-32 were also rejected under 35 U.S.C. § 112, first paragraph, as assertedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that, at the time the application was filed, the inventors had possession of the claimed invention. Partially in view of the amendments to claims 1-5, 10-13, 16-25 and 31-32, applicants respectfully traverse the rejections.

It was thought that there was an insufficient written description for the structure associated with the function of any compound that inhibits binding of free light chain of immunoglobulin (LC) to mast cells, any pharmaceutical composition comprising any peptide, or any compound that is any LC-binding peptide fragment of Tamm-Horsfall glycoprotein or any derivative thereof. It was further asserted that since the specification only discloses one working peptide consisting of SEQ ID NO: 1 that the applicants were not in possession of the claimed genus.

Although applicants do not agree that the claims contain subject matter not described in the specification in such a way to reasonably convey to one skilled in the art that the applicants had possession of the claimed invention, the claims have been amended to a "pharmaceutical composition" comprising a "peptide" instead of being directed to "any compound." Further, the claims are directed to a "peptide" that inhibits the binding of IgLC to mast cells and the ability of

the claimed peptide to inhibit the binding of IgLC to mast cells appears to modulate the role of mast cells as indicated in the *in vivo* experiments disclosed in the as-filed specification. (*See, Id.* at page 9, lines 16-20 and page 10, lines 11-15).

"An applicant may also show that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics which provide evidence that applicant was in possession of the claimed invention." (M.P.E.P. § 2163). Since the specification discloses working examples of peptides with the identifying characteristic that the peptides inhibit the binding of free light chain of immunoglobulin to mast cells, the applicants have shown possession of the claimed invention. (*See, Id.* at Examples 2, 3 and 4, pages 8-10).

With further regard to claim 22, the claim is directed to a pharmaceutical composition including a peptide produced by a process. The process includes screening a series of peptides for each of the peptide's capacity to bind an immunoglobulin's free light chain (LC) and compete with a peptide of SEQ ID NO: 1. Since the process has been described for detecting peptides that bind other proteins, IgLC binding peptides can be selected using the process of claim 22 without undue experimentation. (*See*, Slootstra et al., Structural aspects of antibody-antigen interaction revealed through small random peptide libraries, Mol-Divers 1, pages 87-96 (1996); *See also*, Slootstra et al., Screening of a small set of random peptides: a new strategy to identify synthetic peptides that mimic epitopes, J. Mol. Recongit. 10, pages 217-224 (1997)).

Accordingly, applicants had possession of the claimed invention at the time the application was filed. Reconsideration and withdrawal of the written description rejections of claims 1-5, 10-13, 16-25 and 31-32 are thus requested.

IV. Rejections under 35 U.S.C. § 112, second paragraph

Claims 22-25 stand rejected under 35 U.S.C. § 112, second paragraph, as assertedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. At least partly in view of the amendments to claims 22-25, applicants respectfully traverse the rejections.

Although applicants do not agree that claims 22-25 are indefinite, claim 22 has been amended to recite in part "a labeled peptide, said labeled peptide comprising a peptide and a

label, and said peptide capable of: i) binding the free light chain of immunoglobulin." As amended, it is clear that the "labeled peptide" includes a label and a peptide, wherein the peptide is capable of binding the free light chain of immunoglobulin.

In view of the amendment to claim 22, reconsideration and withdrawal of the indefiniteness rejections of claims 22-25 are respectfully requested.

V. Rejections under 35 U.S.C. § 102(b)

Claims 1-5, 10-13, 16-25 and 31-32 stand rejected under 35 U.S.C. § 102(b) as assertedly being anticipated by Huang et al. (J. Clin. Invest. 99(4): 732-36, 1997). Applicants respectfully traverse the rejections as hereinafter set forth.

The claims are not anticipated since each and every limitation of the claims is not disclosed by Huang et al. As amended, the claims are directed to a "pharmaceutical composition" comprising a "peptide" wherein the peptide inhibits binding of the IgLC to mast cells. The Huang et al. reference does not disclose a pharmaceutical composition. Rather, the peptides of Huang et al. are obtained using tri-fluoro acetic acid. (*See*, Huang et al., page 732 under heading "*Protein and peptide preparations*"). As known by those skilled in the art, tri-fluoro acetic acid is a solvent for which no adequate toxicological data was found at the time the application was filed. If such residual solvents are present in a pharmaceutical product, the manufacturer should justify such use. (*See*, ICH Topic Q3C: Note for Guidance on Impurities: Residual Solvents, page 8 (CPMP/ICH283/95) (attached hereto)).

Further, Huang et al. does not disclose sterile conditions for the production of the peptides as required to produce a pharmaceutical composition and the peptides of Huang et al. are dissolved in PBS for use in an *in vitro* test. (*See*, Huang et al., page 733, col. 1). Thus, Huang et al. does not disclose a pharmaceutical composition since the peptides disclosed in Huang et al. are not sterile and are not prepared for pharmaceutical purposes.

Huang et al. also does not disclose the use of a LC binding peptide for medical treatment, but is limited to the use of a peptide that binds LC to show the presence of LC in nephropathy. (*See*, *Id.* at page 732). Further, the *in vivo* study disclosed in Huang et al. inhibits LC aggregation

in rat kidney by administering colchicine. (*See*, *Id.* at page 736). The use of colchicine indicates that Huang et al. does not use the peptides as a pharmaceutical.

Since Huang et al. does not disclose a pharmaceutical composition as required by the pending claims, the claims are not anticipated. Reconsideration and withdrawal of the anticipation rejections of claims 1-5, 10-13, 16-25 and 31-32 are thus requested.

CONCLUSION

In view of the foregoing amendments and remarks, applicants respectfully submit that the claims define patentable subject matter. If questions remain after consideration of the amendments and remarks presented herein, the Office is kindly requested to contact applicants' attorney at the address or telephone number given herein.

Respectfully submitted,

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Date: January 2, 2003
AFN/afn
Document in ProLaw

Attachments: Letter to the Chief Draftsman
Note for Guidance on Impurities: Residual Solvents (CPMP/ICH/283/95)

MARKED UP VERSION OF SPECIFICATION SHOWING CHANGES MADE

To determine the presence of kappa Ig LC, the hapten-binding proteins were fractionated using 12.5% SDS-PAGE, blotted onto PVDF and tested with horseradish peroxidase-labeled anti-Ig kappa LC (The Binding Site, Birmingham, U.K.) in a dilution of 1:2000. Immunoreactive proteins were visualized using ECL (Amersham Pharmacia Biotech Benelux, Roosendaal, the Netherlands) according to the manufacturer's recommendations (FIG. 2, portion A). This showed that in lymphocyte factors specific for picric acid, dinitrofluorobenzene and exazolon respectively, the presence of [Ic] Ig LC could be demonstrated using an anti-kappa Ig LC-specific antibody.

MARKED UP VERSION OF CLAIMS SHOWING CHANGES MADE

1. (Twice amended) A [compound] pharmaceutical composition comprising a peptide that inhibits binding of free light chain of immunoglobulin (LC) to mast cells:
wherein when the [compound] peptide is in the presence of an equimolar quantity of the free light chain of immunoglobulin in a solution, the free light chain of immunoglobulin's binding to said mast cells is reduced by at least 5%.

2. (Twice amended) The [compound] pharmaceutical composition of claim 1, wherein the [compound] peptide also:
binds to the free light chain of immunoglobulin;
competes with a second peptide for binding to the free light chain of immunoglobulin[,];
wherein said second peptide has the amino acid sequence AHWSGHCCL (SEQ ID NO:1); and
wherein when said [compound] peptide and said second peptide are present in equimolar amounts in a solution, said [compound] peptide reduces binding of said second peptide to said free light chain of immunoglobulin by at least 5%.

3. (Thrice amended) The [compound] pharmaceutical composition of claim 2, wherein the [compound] peptide reduces the binding of said second peptide to the free light chain of immunoglobulin by at least 10%.

4. (Twice amended) The [compound] pharmaceutical composition of claim 2, wherein the [compound] peptide is a peptidomimeticum.

5. (Twice amended) The [compound] pharmaceutical composition of claim [1] 2, wherein the [compound] peptide is a pharmaceutically acceptable compound.

10. (Thrice amended) A [compound] pharmaceutical composition for treating a disease state in a subject, said disease state characterized by exhibiting:

i) a serum concentration of free light chain of immunoglobulin in serum of at least 8 mg/l;

ii) a spinal fluid concentration of free light kappa-chain of immunoglobulin of at least 70 μg/l; and/or

iii) a spinal fluid concentration of free lambda-chain of immunoglobulin of at least 300 μg/l;

said [compound] pharmaceutical composition comprising a peptide,[:] wherein when the [compound] peptide is in the presence of an equimolar quantity of free light chain of immunoglobulin (LC), the peptide reduces the equimolar quantity of LC's binding to mast cells present in solution therewith by at least 5%.

11. (Thrice amended) The [compound] pharmaceutical composition of claim 10, wherein the [compound] peptide inhibits LC's binding to mast cells present in solution by at least 10%.

12. (Thrice amended) The [compound] pharmaceutical composition of claim 10, wherein the disease is selected from the group consisting of asthma, allergy, chronic inflammatory bowel disorders, viral infection and multiple sclerosis.

13. (Thrice amended) A pharmaceutical composition comprising:

a [compound] peptide that, in the presence of an equimolar quantity of free light chain of immunoglobulin (LC), reduces the equimolar quantity of LC's binding to mast cells present in the solution by at least 5%[,]; and

[with] a pharmaceutically acceptable carrier or diluent.

16. (Twice amended) The [compound] pharmaceutical composition of claim 2, wherein the [compound] peptide reduces the binding of said second peptide to the free light chain of immunoglobulin by at least 25%.

17. (Twice amended) The [compound] pharmaceutical composition of claim 2, wherein the [compound] peptide reduces the binding of said second peptide to the free light chain of immunoglobulin by at least 50%.

18. (Twice amended) The [compound] pharmaceutical composition of claim 2, wherein the [compound] peptide reduces the binding of said second peptide to the free light chain of immunoglobulin by at least 75%.

19. (Twice amended) The [compound] pharmaceutical composition of claim 2, wherein the [compound] peptide reduces the binding of said second peptide to the free light chain of immunoglobulin by at least 90%.

20. (Twice amended) The [compound] pharmaceutical composition of claim 4, wherein the [compound] peptide has a mass of less than 10 kDal.

21. (Twice amended) The [compound] pharmaceutical composition of claim 5, wherein the [compound] peptide has a mass of less than 2 kDal.

22. (Twice amended) A [compound] pharmaceutical composition comprising a peptide produced by a process, said process comprising:

screening a series of [compounds] peptides for each of said [compound's] peptide's capability to bind an immunoglobulin's free light chain (LC), said screening comprising:

a) incubating a [compound] peptide from said series of [compounds] peptides with an admixture comprising LC and a labeled [compound] peptide, said labeled [compound] peptide comprising a [compound] peptide and a label, and said [compound] peptide capable of:

i) binding the free light chain of immunoglobulin; and

ii) competing with a second peptide with the amino acid sequence AHWSGHCCL (SEQ ID NO: 1) for binding with the free light chain of immunoglobulin; and

isolating the [compounds] peptides which bind LC and compete with the second peptide.

23. (Twice amended) The [compound] pharmaceutical composition of claim 22, characterized in that the [compound] peptide has a mass less than 10 kDal.

24. (Twice amended) The [compound] pharmaceutical composition of claim 23, wherein the [compound] peptide is an LC-binding peptide fragment of Tamm-Horsfall glycoprotein or a derivative thereof.

25. (Twice amended) The [compound] pharmaceutical composition of claim 22, characterized in that the [compound] peptide has a mass of less than 2 kDal.

31. (Amended) The pharmaceutical composition of claim 13, wherein the [compound] peptide:

binds LC;

competes for binding with LC and a second peptide with the amino acid sequence AHWSGHCCL (SEQ ID NO: 1); and

reduces binding of said second peptide with LC by at least 5% when the [compound] peptide and the second peptide are present in a solution with said LC in equimolar amounts.

32. (Amended) The pharmaceutical composition of claim 13, wherein the [compound] peptide [is a peptide having] has a mass of less than 10 kDal.

Attachments: Marked up Version of Specification and Claims

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MARKED UP VERSION OF SPECIFICATION

Therefore the present invention relates to a compound which inhibits the binding of the free light chain of immunoglobulin to mast cells, wherein the compound, in the presence of an equimolar quantity of free light chain (LC) of immunoglobulin reduces its binding by at least 5%[, said compound not being Tamm-Horsefall glycoprotein (THP), or LC-binding peptide fragments thereof].

MARKED UP VERSION OF CLAIMS

1. (Amended) A compound that inhibits [the] binding of [the] free light chain of immunoglobulin (LC) to mast cells[,]:

wherein [the] when the compound[,] is in the presence of an equimolar quantity of the free light chain of immunoglobulin in a solution, [reduces binding between] the free light chain of immunoglobulin's [and] binding to said mast cells is reduced by at least 5%[, said compound not being Tamm-Horsefall glycoprotein (THP), or LC-binding peptide fragments thereof].

2. (Amended) The compound of claim 1, wherein the compound also:

[can] binds to the free light chain of immunoglobulin;

[and can] competes with a peptide [capable of] for binding to the free light chain of immunoglobulin, wherein said peptide [having] has the amino acid sequence [(]AHWSGHCCL[)] (SEQ ID NO:1)[,]; and

wherein when said compound[, in the presence of an equimolar quantity of] and said peptide are present in equimolar amounts in a solution, said compound reduces binding [between] of said peptide [and] to said free light chain of immunoglobulin by at least 5%.

3. (Twice Amended) The compound of claim [1] 2, wherein the compound reduces the binding [between] of [the] said peptide [and] to the free light chain of immunoglobulin by at least 10%.

4. (Amended) The compound of claim 2, wherein the compound is a peptidomimeticum.

5. (Amended) The compound of claim 1, wherein the compound is a pharmaceutically acceptable compound.

10. (Twice Amended) A compound for [use in] treating a disease state in a subject, said disease state characterized by exhibiting [symptoms comprising]:

i) a serum concentration of [the] free light chain of immunoglobulin in serum of at least 8 mg/l; [and/or]

ii) a spinal fluid concentration of [the] free light kappa-chain of immunoglobulin [in spinal fluid] of at least 70 μg/l; and/or

iii) a spinal fluid concentration of [the] free lambda-chain of immunoglobulin [in spinal fluid] of at least 300 μg/l[,];

said compound comprising:

wherein when the compound is in the presence of an equimolar quantity of free light chain of immunoglobulin (LC), reduces the equimolar quantity of LC's binding to mast cells present in solution therewith by at least 5%.

[said drug comprising a compound according to claim 1.]

11. (Twice Amended) The [drug of] compound of claim 10, wherein the compound [is a peptide or peptidomimeticum with a mass of less than] inhibits LC's binding to mast cells present in solution by at least 10% [kDal].

12. (Twice Amended) The [drug] compound of claim 10, wherein the disease is selected from the group consisting of asthma, allergy, chronic inflammatory bowel disorders, viral infection and multiple sclerosis.

13. (Twice Amended) A pharmaceutical composition comprising a compound [selected from the group consisting of a compound that inhibits the binding of the free light chain of

immunoglobulin to mast cells, wherein the compound,] that, in the presence of an equimolar quantity of [the] free light chain of immunoglobulin (LC), reduces the equimolar quantity of LC's binding [between the free light chain of immunoglobulin and said] to mast cells present in the solution by at least 5%, [Tamm-Horsefall glycoprotein (THP) and LC-binding peptides thereof together] with a pharmaceutically acceptable carrier or [excipient] diluent.

16. (Amended) The compound of claim [1] 2, wherein the compound reduces the binding [between] of said [the] peptide [and] to the free light chain of immunoglobulin by at least 25%.

17. (Amended) The compound of claim [1] 2, wherein the compound reduces the binding [between] of said [the] peptide [and] to the free light chain of immunoglobulin by at least 50%.

18. (Amended) The compound of claim [1] 2, wherein the compound reduces the binding [between] of said [the] peptide [and] to the free light chain of immunoglobulin by at least 75%.

19. (Amended) The compound of claim [1] 2, wherein the compound reduces the binding [between] of said [the] peptide [and] to the free light chain of immunoglobulin by at least 90%.

20. (Amended) The compound of [according to] claim [3] 4, wherein the compound [is a peptidomimeticum] has a mass of less than 10 kDal.

21. (Amended) The [drug] compound of claim [11] 5, wherein the compound [is a peptide or peptidomimeticum with] has a mass of less than 2 kDal.

22. (Amended) A compound produced by [the] a process, said process comprising:

screening a series of compounds [based on] for each of said compound's [ability] capability to bind [the] an immunoglobulin's free light chain (LC), [of immunoglobulin, wherein] said screening compris[es]ing:

a) [using a labeled] incubating a compound from said series of compounds with an admixture comprising LC and a labeled compound, said labeled compound comprising a compound and a label, and said compound capable of:

i) binding the free light chain of immunoglobulin; and

ii) [capable of] competing with a peptide with the amino acid sequence AHWSGHCCL (SEQ ID NO: 1) for binding [to] with the free light chain of immunoglobulin; and

isolating the compounds which bind LC and compete with the peptide.

[performing a test comprising a competition reaction between at least one compound of said series of compounds and said peptide for binding to the light chain of immunoglobulin; and selecting a compound from said series of compounds that inhibits binding between the peptide and the light chain of immunoglobulin.]

23. (Amended) The compound of claim 22, [wherein the compound is a peptide or peptidomimeticum] characterized in that the compound has a mass less than 10 kDal.

24. (Amended) The compound of claim 23, wherein the compound [has a mass of less than 10 kDal] is an LC-binding peptide fragment of Tamm-Horsfall glycoprotein or a derivative thereof.

25. (Amended) The compound of claim [23] 22, [wherein the] characterized in that the compound has a mass of less than 2 kDal.

The European Agency for the Evaluation of Medicinal Products
Human Medicines Evaluation Unit

ICH Topic Q 3 C
Impurities: Residual Solvents

Step 4, Consensus guideline, 17 July 1997

NOTE FOR GUIDANCE ON IMPURITIES: RESIDUAL SOLVENTS (CPMP/ICH/283/95)

TRANSMISSION TO CPMP	November 1996
TRANSMISSION TO INTERESTED PARTIES	November 1996
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IMPURITIES: RESIDUAL SOLVENTS

ICH Harmonised Tripartite Guideline

Table of Contents

1. INTRODUCTION

The objective of this guideline is to recommend acceptable amounts for residual solvents in pharmaceuticals for the safety of the patient. The guideline recommends use of less toxic solvents and describes levels considered to be toxicologically acceptable for some residual solvents.

Residual solvents in pharmaceuticals are defined here as organic volatile chemicals that are used or produced in the manufacture of active substances or excipients, or in the preparation of medicinal products. The solvents are not completely removed by practical manufacturing techniques. Appropriate selection of the solvent for the synthesis of active substance may enhance the yield, or determine characteristics such as crystal form, purity, and solubility. Therefore, the solvent may sometimes be a critical parameter in the synthetic process. This guideline does not address solvents deliberately used as excipients nor does it address solvates. However, the content of solvents in such products should be evaluated and justified.

Since there is no therapeutic benefit from residual solvents, all residual solvents should be removed to the extent possible to meet product specifications, good manufacturing practices, or other quality-based requirements. Medicinal products should contain no higher levels of residual solvents than can be supported by safety data. Some solvents that are known to cause unacceptable toxicities (Class 1, Table 1) should be avoided in the production of active substances, excipients, or medicinal products unless their use can be strongly justified in a risk-benefit assessment. Some solvents associated with less severe toxicity (Class 2, Table 2) should be limited in order to protect patients from potential adverse effects. Ideally, less toxic solvents (Class 3, Table 3) should be used where practical. The complete list of solvents included in this guideline is given in Appendix 1.

The lists are not exhaustive and other solvents can be used and later added to the lists. Recommended limits of Class 1 and 2 solvents or classification of solvents may change as new safety data becomes available. Supporting safety data in a marketing application for a new medicinal product containing a new solvent may be based on concepts in this guideline or the concept of qualification of impurities as expressed in the guideline for active substance (Q3A Impurities in New Active Substances) or medicinal product (Q3B, Impurities in New Medicinal Products), or all three guidelines.

2. SCOPE OF THE GUIDELINE

Residual solvents in active substances, excipients, and in medicinal products are within the scope of this guideline. Therefore, testing should be performed for residual solvents when production or purification processes are known to result in the presence of such solvents. It is only necessary to test for solvents that are used or produced in the manufacture or purification of medicinal substances, excipients, or medicinal product. Although manufacturers may choose to test the medicinal product, a cumulative method may be used to calculate the residual solvent levels in the product from the levels in the ingredients used to produce the product. If the calculation results in a level equal to or below that recommended in this guideline, no testing of the medicinal product for residual solvents need be considered. If, however, the calculated level is above the recommended level, the medicinal product should be

tested to ascertain whether the formulation process has reduced the relevant solvent level to within the acceptable amount. The medicinal product should also be tested if a solvent is used during its manufacture.

This guideline does not apply to potential new active substances, excipients, or medicinal products used during the clinical research stages of development, nor does it apply to existing marketed medicinal products.

The guideline applies to all dosage forms and routes of administration. Higher levels of residual solvents may be acceptable in certain cases such as short term (30 days or less) or topical application. Justification for these levels should be made on a case by case basis.

See Appendix 2 for additional background information related to residual solvents.

3. GENERAL PRINCIPLES

3.1 Classification of Residual Solvents by Risk Assessment

The term "tolerable daily intake" (TDI) is used by the International Program on Chemical Safety (IPCS) to describe exposure limits of toxic chemicals and "acceptable daily intake" (ADI) is used by the World Health Organisation (WHO) and other national and international health authorities and institutes. The new term "permitted daily exposure" (PDE) is defined in the present guideline as a pharmaceutically acceptable intake of residual solvents to avoid confusion of differing values for ADIs of the same substance.

Residual solvents assessed in this guideline are listed in Appendix 1 by common names and structures. They were evaluated for their possible risk to human health and placed into one of three classes as follows:

Class 1 solvents: Solvents to be avoided

Known human carcinogens, strongly suspected human carcinogens, and environmental hazards.

Class 2 solvents: Solvents to be limited

Non-genotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratogenicity.

Solvents suspected of other significant but reversible toxicities.

Class 3 solvents: Solvents with low toxic potential

Solvents with low toxic potential to man; no health-based exposure limit is needed. Class 3 solvents have PDEs of 50 mg or more per day.

3.2 Methods for Establishing Exposure Limits

The method used to establish permitted daily exposures for residual solvents is presented in Appendix 3. Summaries of the toxicity data that were used to establish limits are published in Pharmeuropa, Vol. 9, No. 1, Supplement, April 1997.

3.3 Options for Describing Limits of Class 2 Solvents

Two options are available when setting limits for Class 2 solvents.

Option 1: The concentration limits in ppm stated in Table 2 can be used. They were calculated using equation (1) below by assuming a product mass of 10 g administered daily.

$$(1): \qquad \text{Concentration (ppm)} = \frac{1000 \times \text{PDE}}{\text{dose}}$$

Here, PDE is given in terms of mg/day and dose is given in g/day.

These limits are considered acceptable for all substances, excipients, or products. Therefore this option may be applied if the daily dose is not known or fixed. If all excipients and active substances in a formulation meet the limits given in Option 1, then these components may be used in any proportion. No further calculation is necessary provided the daily dose does not exceed 10 g. Products that are administered in doses greater than 10 g per day should be considered under Option 2.

Option 2: it is not considered necessary for each component of the medicinal product to comply with the limits given in Option 1. The PDE in terms of mg/day as stated in Table 2 can be used with the known maximum daily dose and equation (1) above to determine the concentration of residual solvent allowed in the medicinal product. Such limits are considered acceptable provided that it has been demonstrated that the residual solvent has been reduced to the practical minimum. The limits should be realistic in relation to analytical precision, manufacturing capability, reasonable variation in the manufacturing process, and the limits should reflect contemporary manufacturing standards.

Option 2 may be applied by adding the amounts of a residual solvent present in each of the components of the medicinal product. The sum of the amounts of solvent per day should be less than that given by the PDE.

Consider an example of the use of Option 1 and Option 2 applied to acetonitrile in a medicinal product. The permitted daily exposure to acetonitrile is 4.1 mg per day; thus, the Option 1 limit is 410 ppm. The maximum administered daily mass of a medicinal product is 5.0 g, and the medicinal product contains two excipients. The composition of the medicinal product and the calculated maximum content of residual acetonitrile are given in the following table.

Component	Amount in formulation	Acetonitrile content	Daily exposure
Active substance	0.3 g	800 ppm	0.24 mg
Excipient 1	0.9 g	400 ppm	0.36 mg
Excipient 2	3.8 g	800 ppm	3.04 mg
Medicinal product	5.0 g	728 ppm	3.64 mg

Excipient 1 meets the Option 1 limit, but the active substance, excipient 2, and the medicinal product do not meet the Option 1 limit. Nevertheless, the product meets the Option 2 limit of 4.1 mg per day and thus conforms to the recommendations in this guideline.

Consider another example using acetonitrile as residual solvent. The maximum administered daily mass of a medicinal product is 5.0 g, and the medicinal product contains two excipients. The composition of the medicinal product and the calculated maximum content of residual acetonitrile is given in the following table.

Component	Amount in formulation	Acetonitrile content	Daily exposure
Active substance	0.3 g	800 ppm	0.24 mg
Excipient 1	0.9 g	2000 ppm	1.80 mg
Excipient 2	3.8 g	800 ppm	3.04 mg
Medicinal product	5.0 g	1016 ppm	5.08 mg

In this example, the product meets neither the Option 1 nor the Option 2 limit according to this summation. The manufacturer could test the product to determine if the formulation process reduced the level of acetonitrile. If the level of acetonitrile was not reduced during formulation to the allowed limit, then the manufacturer of the product should take other steps to reduce the amount of acetonitrile in the product. If all of these steps fail to reduce the level of residual solvent, in exceptional cases the manufacturer could provide a summary of efforts made to reduce the solvent level to meet the guideline value, and provide a risk-benefit analysis to support allowing the product to be utilised with residual solvent at a higher level.

3.4 Analytical Procedures

Residual solvents are typically determined using chromatographic techniques such as gas chromatography, Any harmonised procedures for determining levels of residual solvents as described in the pharmacopoeias should be used, if feasible. Otherwise, manufacturers would be free to select the most appropriate validated analytical procedure for a particular application. If only Class 3 solvents are present, a non-specific method such as loss on drying may be used.

Validation of methods for residual solvents should conform to the ICH guidelines "Validation of analytical procedures: definition and terminology" and "Validation of analytical procedures: methodology."

3.5 Reporting Levels of Residual Solvents

Manufacturers of pharmaceutical products need certain information about the content of residual solvents in excipients or active substances in order to meet the criteria of this guideline. The following statements are given as acceptable examples of the information that could be provided from a supplier of excipients or active substances to a pharmaceutical manufacturer. The supplier might choose one of the following as appropriate:

- Only Class 3 solvents are likely to be present. Loss on drying is less than 0.5%.
- Only Class 2 solvents X, Y, ... are likely to be present. All are below the Option 1 limit. (Here the supplier would name the Class 2 solvents represented by X, Y, ...)
- Only Class 2 solvents X, Y, ... and Class 3 solvents are likely to be present. Residual Class 2 solvents are below the Option 1 limit and residual Class 3 solvents are below 0.5%.

If Class 1 solvents are likely to be present, they should be identified and quantified.

"Likely to be present" refers to the solvent used in the final manufacturing step and to solvents that are used in earlier manufacturing steps and not removed consistently by a validated process.

If solvents of Class 2 or Class 3 are present at greater than their Option 1 limits or 0.5%, respectively, they should be identified and quantified.

4. LIMITS OF RESIDUAL SOLVENTS

4.1 Solvents to be Avoided

Solvents in Class 1 should not be employed in the manufacture of active substances, excipients, and medicinal products because of their unacceptable toxicity or their deleterious environmental effect. However, if their use is unavoidable in order to produce a medicinal product with a significant therapeutic advance, then their levels should be restricted as shown in Table 1, unless otherwise justified. 1,1,1-Trichloroethane is included in Table 1 because it is an environmental hazard. The stated limit of 1500 ppm is based on a review of the safety data.

Table 1: Class 1 Solvents in pharmaceutical products (solvents that should be avoided)

Solvent	Concentration Limit (ppm)	Concern
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

4.2 Solvents to be Limited

Solvents in Table 2 should be limited in pharmaceutical products because of their inherent toxicity. PDEs are given to the nearest 0.1 mg/day, and concentrations are given to the nearest 10 ppm. The stated values do not reflect the necessary analytical precision of determination. Precision should be determined as part of the validation of the method.

Table 2: Class 2 Solvents in Pharmaceutical Products

Solvent	PDE (mg/day)	Concentration Limit (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cyclohexane	38.8	3880
1,2-Dichloroethene	18.7	1870
Dichloromethane	6.0	600
1,2-Dimethoxyethane	1.0	100
N,N-Dimethylacetamide	10.9	1090
N,N-Dimethylformamide	8.8	880
1,4-Dioxane	3.8	380
2-Ethoxyethanol	1.6	160
Ethylene glycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	30.0	3000
2-Methoxyethanol	0.5	50
Methylbutylketone	0.5	50
Methylcyclohexane	11.8	1180
N-Methylpyrrolidone	48.4	4840
Nitromethane	0.5	50
Pyridine	2.0	200
Sulfolane	1.6	160
Tetralin	1.0	100
Toluene	8.9	890
1,1,2-Trichloroethene	0.8	80
Xylene*	21.7	2170

* usually 60% m-xylene, 14% p-xylene, 9% o-xylene with 17% ethyl benzene.

4.3 Solvents with Low Toxic Potential

Solvents in Class 3 (shown in Table 3) may be regarded as less toxic and of lower risk to human health. Class 3 includes no solvent known as a human health hazard at levels normally accepted in pharmaceuticals. However, there are no long-term toxicity or carcinogenicity

studies for many of the solvents in Class 3. Available data indicate that they are less toxic in acute or short-term studies and negative in genotoxicity studies. It is considered that amounts of these residual solvents of 50 mg per day or less (corresponding to 5000 ppm or 0.5% under Option 1) would be acceptable without justification. Higher amounts may also be acceptable provided they are realistic in relation to manufacturing capability and good manufacturing practice.

Table 3: Class 3 Solvents which should be limited by GMP or other quality-based requirements

Acetic acid	Heptane
Acetone	Isobutyl acetate
Anisole	Isopropyl acetate
1-Butanol	Methyl acetate
2-Butanol	3-Methyl-1-butanol
Butyl acetate	Methylethyl ketone
tert-Butylmethyl ether	Methylisobutyl ketone
Cumene	2-Methyl-1-propanol
Dimethylsulfoxide	Pentane
Ethanol	1-Pentanol
Ethyl acetate	1-Propanol
Ethyl ether	2-Propanol
Ethyl formate	Propyl acetate
Formic acid	Tetrahydrofuran

4.4 Solvents For Which No Adequate Toxicological Data Was Found

The following solvents (Table 4) may also be of interest to manufacturers of excipients, active substances, or medicinal products. However, no adequate toxicological data on which to base a PDE was found. Manufacturers should supply justification for residual levels of these solvents in pharmaceutical products.

Table 4: Solvents for which no adequate Toxicological Data was found

1,1-Diethoxypropane	Methylisopropyl ketone
1,1-Dimethoxymethane	Methyltetrahydrofuran
2,2-Dimethoxypropane	Petroleum ether
Isooctane	Trichloroacetic acid
Isopropyl ether	Trifluoroacetic acid

GLOSSARY

Genotoxic carcinogens: Carcinogens which produce cancer by affecting genes or chromosomes.

LOEL: Abbreviation for lowest-observed effect level.

Lowest-observed effect level: The lowest dose of substance in a study or group of studies that produces biologically significant increases in frequency or severity of any effects in the exposed humans or animals.

Modifying factor: A factor determined by professional judgement of a toxicologist and applied to bioassay data to relate that data safely to humans.

Neurotoxicity: The ability of a substance to cause adverse effects on the nervous system.

NOEL: Abbreviation for no-observed-effect level.

No-observed-effect level: The highest dose of substance at which there are no biologically significant increases in frequency or severity of any effects in the exposed humans or animals.

PDE: Abbreviation for permitted daily exposure.

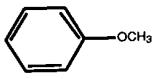
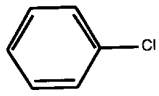
Permitted daily exposure: The maximum acceptable intake per day of residual solvent in pharmaceutical products.

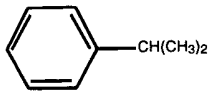
Reversible toxicity: The occurrence of harmful effects that are caused by a substance and which disappear after exposure to the substance ends.

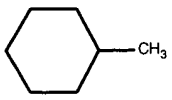
Strongly suspected human carcinogen: A substance for which there is no epidemiological evidence of carcinogenesis but there are positive genotoxicity data and clear evidence of carcinogenesis in rodents.

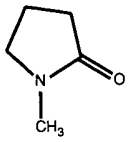
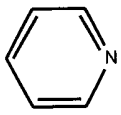
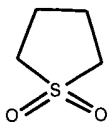
Teratogenicity: The occurrence of structural malformations in a developing fetus when a substance is administered during pregnancy.

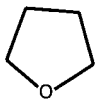
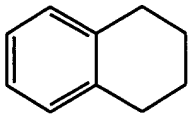
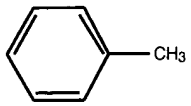
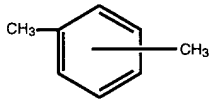
APPENDIX 1: LIST OF SOLVENTS INCLUDED IN THE GUIDELINE

Solvent	Other Names	Structure	Class
Acetic acid	Ethanoic acid	CH_3COOH	Class 3
Acetone	2-Propanone Propan-2-one	CH_3COCH_3	Class 3
Acetonitrile		CH_3CN	Class 2
Anisole	Methoxybenzene		Class 3
Benzene	Benzol		Class 1
1-Butanol	*n*-Butyl alcohol Butan-1-ol	$CH_3(CH_2)_3OH$	Class 3
2-Butanol	*sec*-Butyl alcohol Butan-2-ol	$CH_3CH_2CH(OH)CH_3$	Class 3
Butyl acetate	Acetic acid butyl ester	$CH_3COO(CH_2)_3CH_3$	Class 3
tert-Butylmethyl ether	2-Methoxy-2-methyl-propane	$(CH_3)_3COCH_3$	Class 3
Carbon tetrachloride	Tetrachloromethane	CCl_4	Class 1
Chlorobenzene			Class 2
Chloroform	Trichloromethane	$CHCl_3$	Class 2

Cumene	Isopropylbenzene (1-Methyl)ethylbenzene		Class 3
Cyclohexane	Hexamethylene		Class 2
1,2-Dichloroethane	*sym*-Dichloroethane Ethylene dichloride Ethylene chloride	CH_2ClCH_2Cl	Class 1
1,1-Dichloroethene	1,1-Dichloroethylene Vinylidene chloride	$H_2C=CCl_2$	Class 1
1,2-Dichloroethene	1,2-Dichloroethylene Acetylene dichloride	$ClHC=CHCl$	Class 2
Dichloromethane	Methylene chloride	CH_2Cl_2	Class 2
1,2-Dimethoxyethane	Ethyleneglycol dimethyl ether Monoglyme Dimethyl Cellosolve	$H_3COCH_2CH_2OCH_3$	Class 2
N,N-Dimethylacetamide	DMA	$CH_3CON(CH_3)_2$	Class 2
N,N-Dimethylformamide	DMF	$HCON(CH_3)_2$	Class 2
Dimethyl sulfoxide	Methylsulfinylmethane Methyl sulfoxide DMSO	$(CH_3)_2SO$	Class 3
1,4-Dioxane	p-Dioxane [1,4]Dioxane		Class 2
Ethanol	Ethyl alcohol	CH_3CH_2OH	Class 3
2-Ethoxyethanol	Cellosolve	$CH_3CH_2OCH_2CH_2OH$	Class 2

Ethyl acetate	Acetic acid ethyl ester	$CH_3COOCH_2CH_3$	Class 3
Ethyleneglycol	1,2-Dihydroxyethane 1,2-Ethanediol	$HOCH_2CH_2OH$	Class 2
Ethyl ether	Diethyl ether Ethoxyethane 1,1'-Oxybisethane	$CH_3CH_2OCH_2CH_3$	Class 3
Ethyl formate	Formic acid ethyl ester	$HCOOCH_2CH_3$	Class 3
Formamide	Methanamide	$HCONH_2$	Class 2
Formic acid		$HCOOH$	Class 3
Heptane	n-Heptane	$CH_3(CH_2)_5CH_3$	Class 3
Hexane	n-Hexane	$CH_3(CH_2)_4CH_3$	Class 2
Isobutyl acetate	Acetic acid isobutyl ester	$CH_3COOCH_2CH(CH_3)_2$	Class 3
Isopropyl acetate	Acetic acid isopropyl ester	$CH_3COOCH(CH_3)_2$	Class 3
Methanol	Methyl alcohol	CH_3OH	Class 2
2-Methoxyethanol	Methyl Cellosolve	$CH_3OCH_2CH_2OH$	Class 2
Methyl acetate	Acetic acid methyl ester	CH_3COOCH_3	Class 3
3-Methyl-1-butanol	Isoamyl alcohol Isopentyl alcohol 3-Methylbutan-1-ol	$(CH_3)_2CHCH_2CH_2OH$	Class 3
Methylbutyl ketone	2-Hexanone Hexan-2-one	$CH_3(CH_2)_3COCH_3$	Class 2
Methylcyclohexane	Cyclohexylmethane		Class 2

Methylethyl ketone	2-Butanone MEK Butan-2-one	$CH_3CH_2COCH_3$	Class 3
Methylisobutyl ketone	4-Methylpentan-2-one 4-Methyl-2-pentanone MIBK	$CH_3COCH_2CH(CH_3)_2$	Class 3
2-Methyl-1-propanol	Isobutyl alcohol 2-Methylpropan-1-ol	$(CH_3)_2CHCH_2OH$	Class 3
N-Methylpyrrolidone	1-Methylpyrrolidin-2-one 1-Methyl-2-pyrrolidinone		Class 2
Nitromethane		CH_3NO_2	Class 2
Pentane	*n*-Pentane	$CH_3(CH_2)_3CH_3$	Class 3
1-Pentanol	Amyl alcohol Pentan-1-ol Pentyl alcohol	$CH_3(CH_2)_3CH_2OH$	Class 3
1-Propanol	Propan-1-ol Propyl alcohol	$CH_3CH_2CH_2OH$	Class 3
2-Propanol	Propan-2-ol Isopropyl alcohol	$(CH_3)_2CHOH$	Class 3
Propyl acetate	Acetic acid propyl ester	$CH_3COOCH_2CH_2CH_3$	Class 3
Pyridine			Class 2
Sulfonane	Tetrahydrothiophene 1,1-dioxide		Class 2

Tetrahydrofuran	Tetramethylene oxide Oxacyclopentane		Class 3
Tetralin	1,2,3,4-Tetrahydro-naphthalene		Class 2
Toluene	Methylbenzene		Class 2
1,1,1-Trichloroethane	Methylchlororoform	CH_3CCl_3	Class 1
1,1,2-Trichloroethene	Trichloroethene	$HClC=CCl_2$	Class 2
Xylene*	Dimethybenzene Xylol		Class 2

* usually 60 % m-xylene, 14 % p-xylene, 9 % o-xylene with 17 % ethyl benzene

APPENDIX 2: ADDITIONAL BACKGROUND

A2.1 Environmental Regulation of Organic Volatile Solvents

Several of the residual solvents frequently used in the production of pharmaceuticals are listed as toxic chemicals in Environmental Health Criteria (EHC) monographs and the Integrated Risk information System (IRIS). The objectives of such groups as the International Programme on Chemical Safety (IPCS), the United States Environmental Protection Agency (USEPA), and the United States Food and Drug Administration (USFDA) include the determination of acceptable exposure levels. The goal is protection of human health and maintenance of environmental integrity against the possible deleterious effects of chemicals resulting from long-term environmental exposure. The methods involved in the estimation of maximum safe exposure limits are usually based on tong-term studies. When long-term study data are unavailable, shorter term study data can be used with modification of the approach such as use of larger safety factors. The approach described therein relates primarily to long-term or *life-time exposure of the general population* in the ambient environment, i.e. ambient air, food, drinking water and other media.

A2.2 Residual Solvents in Pharmaceuticals

Exposure limits in this guideline are established by referring to methodologies and toxicity data described in EHC and IRIS monographs. However, some specific assumptions about residual solvents to be used in the synthesis and formulation of pharmaceutical products should be taken into account in establishing exposure limits. They are:

1. Patients (not the general population) use pharmaceuticals to treat their diseases or for prophylaxis to prevent infection or disease.
2. The assumption of life-time patient exposure is not necessary for most pharmaceutical products but may be appropriate as a working hypothesis to reduce risk to human health.
3. Residual solvents are unavoidable components in pharmaceutical production and will often be a part of medicinal products.
4. Residual solvents should not exceed recommended levels except in exceptional circumstances.
5. Data from toxicological studies that are used to determine acceptable levels for residual solvents should have been generated using appropriate protocols such as those described for example by OECD, EPA. and the FDA Red Book.

APPENDIX 3: METHODS FOR ESTABLISHING EXPOSURE LIMITS

The Gaylor-Kodell method of risk assessment (Gaylor, D. W. and Kodell, R. L.: Linear Interpolation algorithm for low dose assessment of toxic substance. J Environ. Pathology, 4, 305, 1980) is appropriate for Class 1 carcinogenic solvents. Only in cases where reliable carcinogenicity data are available should extrapolation by the use of mathematical models be applied to setting exposure limits. Exposure limits for Class 1 solvents could be determined with the use of a large safety factor (i.e., 10,000 to 100,000) with respect to the no-observed-effect level (NOEL). Detection and quantitation of these solvents should be by state-of-the-art analytical techniques.

Acceptable exposure levels in this guideline for Class 2 solvents were established by calculation of PDE values according to the procedures for setting exposure limits in pharmaceuticals (Pharmacopeial Forum, Nov-Dec 1989), and the method adopted by IPCS for Assessing Human Health Risk of Chemicals (Environmental Health Criteria 170, WHO, 1994). These methods are similar to those used by the USEPA (IRIS) and the USFDA (Red Book) and others. The method is outlined here to give a better understanding of the origin of the PDE values. It is not necessary to perform these calculations in order to use the PDE values tabulated in Section 4 of this document.

PDE is derived from the no-observed-effect level (NOEL), or the lowest-observed effect level (LOEL) in the most relevant animal study as follows:

$$PDE = \frac{NOEL \times Weight\ Adjustment}{F1 \times F2 \times F3 \times F4 \times F5}$$

The PDE is derived preferably from a NOEL. If no NOEL is obtained, the LOEL may be used. Modifying factors proposed here, for relating the data to humans, are the same kind of "uncertainty factors" used in Environmental Health Criteria (Environmental Health Criteria 170, World Health Organisation, Geneva, 1994), and "modifying factors" or "safety factors" in Pharmacopeial Forum. The assumption of 100% systemic exposure is used in all calculations regardless of route of administration.

The modifying factors are as follows:

F1 = A factor to account for extrapolation between species

F1 = 5 for extrapolation from rats to humans

F1 = 12 for extrapolation from mice to humans

F1 = 2 for extrapolation from dogs to humans

F1 = 2.5 for extrapolation from rabbits to humans

F1 = 3 for extrapolation from monkeys to humans

F1 = 10 for extrapolation from other animals to humans

F1 takes into account the comparative surface area:body weight ratios for the species concerned and for man. Surface area (S) is calculated as:

$$S = kM^{0.67}$$

in which M = body mass, and the constant k has been taken to be 10. The body weights used in the equation are those shown below in Table A3.1.

F2 = A factor of 10 to account for variability between individuals

A factor of 10 is generally given for all organic solvents, and 10 is used consistently in this guideline.

F3 = A variable factor to account for toxicity studies of short-term exposure

F3 = 1 for studies that last at least one half lifetime (1 year for rodents or rabbits; 7 years for cats, dogs and monkeys).

F3 = 1 for reproductive studies in which the whole period of organogenesis is covered.

F3 = 2 for a 6-month study in rodents, or a 3.5-year study in non-rodents.

F3 = 5 for a 3-month study in rodents, or a 2-year study in non-rodents.

F3 = 10 for studies of a shorter duration.

In all cases, the higher factor has been used for study durations between the time points, e.g. a factor of 2 for a 9-month rodent study.

F4 = A factor that may be applied in cases of severe toxicity, e.g. non-genotoxic carcinogenicity, neurotoxicity or teratogenicity. In studies of reproductive toxicity, the following factors are used:

F4 = 1 for fetal toxicity associated with maternal toxicity

F4 = 5 for fetal toxicity without maternal toxicity

F4 = 5 for a teratogenic effect with maternal toxicity

F4 = 10 for a teratogenic effect without maternal toxicity

F5 = A variable factor that may be applied if the no-effect level was not established

When only an LOEL is available, a factor of up to 10 could be used depending on the severity of the toxicity.

The weight adjustment assumes an arbitrary adult human body weight for either sex of 50 kg. This relatively low weight provides an additional safety factor against the standard weights of 60 kg or 70 kg that are often used in this type of calculation. It is recognised that some adult patients weigh less than 50 kg; these patients are considered to be accommodated by the built-in safety factors used to determine a PDE. If the solvent was present in a formulation specifically intended for paediatric use, an adjustment for a lower body weight would be appropriate.

As an example of the application of this equation, consider a toxicity study of acetonitrile in mice that is summarised in Pharmeuropa, Vol. 9, No. 1, Supplement, April 1997, page S24. The NOEL is calculated to be 50.7 mg kg^{-1} day^{-1}. The PDE for acetonitrile in this study is calculated as follows:

$$PDE = \frac{50.7\ mgkg^{-1}day^{-1} \times 50\ kg}{12 \times 10 \times 5 \times 1 \times 1} = 4.22 mg.day^{-1}$$

In this example,

F1 = 12 to account for the extrapolation from mice to humans
F2 = 10 to account for differences between individual humans
F3 = 5 because the duration of the study was only 13 weeks
F4 = 1 because no severe toxicity was encountered
F5 = 1 because the no effect level was determined

Table A.3.1: Values used in the calculations in this document

rat body weight	425g	mouse respiratory volume	43 L/day
pregnant rat body weight	330g	rabbit respiratory volume	1440 L/day
mouse body weight	28g	guinea pig respiratory volume	430 L/day
pregnant mouse body weight	30g	human respiratory volume	28,800L/day
guinea pig body weight	500g	dog respiratory volume	9,000 L/day
Rhesus monkey body weight	2.5kg	monkey respiratory volume	1,150 L/day
Rabbit body weight (pregnant or not)	4kg	mouse water consumption	5 mL
beagle dog body weight	11.5 kg	rat water consumption	30 mL/day
rat respiratory volume	290 L/day	rat food consumption	30 g/day

The equation for an ideal gas, PV = nRT, is used to convert concentrations of gases used in inhalation studies from units of ppm to units of mg/L or mg/m^3. Consider as an example the rat reproductive toxicity study by inhalation of carbon tetrachloride (molecular weight 153.84) is summarised in Pharmeuropa, Vol, 9, No. 1, Supplement, April 1997, page S9.

$$\frac{n}{V} = \frac{P}{RT} = \frac{300 \times 10^{-6}\text{atm} \times 153840 \text{ mg mol}^{-1}}{0.082 \text{ L atm K}^{-1} \text{ mol}^{-1} \times 298 \text{ K}} = \frac{46.15 \text{ mg}}{24.45\text{L}} = 1.89 \text{ mg/L}$$

The relationship 1000 L = 1 m^3 is used to convert to mg/ m^3.